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Hydroxy–PAH levels in atmospheric PM_{10} aerosol samples correlated with season, physical factors and chemical indicators of pollution

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ABSTRACT

This study describes a simple, sensitive method to determine the hydroxy–PAHs, 1–hydroxypyrene (1–OHPYR) and 2–hydroxyphenantrene (2–OHPH), along with the optimization and successful application of the procedure to the analysis of atmospheric aerosol samples. PM_{10} airborne particles were collected on Whatman glass fiber filters and then ultrasonically extracted into methanol. The extracts were filtered and the solvent evaporated under nitrogen flow. The resulting residues were resuspended in 1.0 mL of methanol and directly injected into an HPLC–fluorescence detection system (HPLC–FD) applying the optimal experimental conditions predicted by the optimization procedure. Clean–up of the extracts was not necessary. Using this method, hydroxy–PAHs detected at pg m^{-3} levels in urban aerosol samples obtained over a full year were characterized and the effects of physical (temperature, atmospheric pressure, relative humidity and solar radiation) and chemical factors (O_3, NO, NO_2, N_2O_x and SO_2) were also evaluated. Results indicate an impact of season, with highest concentrations detected in winter (October to February) and lowest in summer (May to September). Through multivariate analysis (factor and cluster analysis) correlations were identified between the levels of these compounds and several indicators of atmospheric pollution.

Keywords:
OH–PAHs
Atmospheric aerosol
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1. Background

Polycyclic aromatic hydrocarbons (PAHs) are potent atmo–spheric pollutants that form when two or more benzene rings fuse together (Liu et al., 2007; Haritash and Kaushik, 2009) as the result of the incomplete combustion of organic matter arising from natural or anthropogenic sources (Williams and Besler, 1994; Mastral et al., 2003). In the atmosphere, PAHs are either present as gasses or retained by particles, depending on their vapor pressure. Both forms come into contact with living organisms and have been linked to several health risks, being classified as mutagens or carcinogens (Petry et al., 1996; WHO, 2003; ATSDR, 2009), according to the International Agency for Research on Cancer (IARC, 2008).

PAHs undergo degradation and several reactions (photo–chemical, with volatile co–pollutants, etc. (Kakimoto et al., 2001; Miet et al., 2009) to give rise to substituted PAHs. Hydroxy–PAHs (OH–PAHs) are generally less volatile than the parent PAHs and tend to be more particle associated (Vione et al., 2004). In the atmosphere, OH–PAHs can be produced as a result of direct emission, e.g. combustion sources, diesel engines, and/or formation in the atmosphere by photo–oxidation of parent PAHs (Atkinson et al., 1987; Atkinson and Arey, 1994; Mallakin et al., 2000). OH–PAHs can be formed through hydroxyl radical addition to the PAH and nitro–PAHs can be produced by reaction of OH–PAHs with nitrogen dioxide and loss of a water molecule (Atkinson et al., 1987; WHO, 2003).

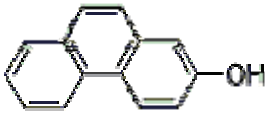
In aerosol samples, these derivatives have been the subject of intense study because of their toxic effects on human health, and nitro– and oxy–PAHs have been found at pg m^{-3} levels (Galceran and Moyano, 1995; Galceran et al., 1995; Kishikawa et al., 2004; Wang et al., 2007). Hydroxy–PAHs seem to show greater cyto–toxicity than their parent PAHs because they can form adducts with DNA (Finlayson–Pitts and Pitts, 2000; Romanoff et al., 2006; Umbuzeiro et al., 2008; Dong and Lee, 2009; Hayakawa, 2009). In addition, OH–PAHs can act as estrogen receptors and show both estrogenic and anti–estrogenic activity because of their structural similarities to steroid hormones (Tran et al., 1996; Hirose et al., 2001). Further, as established metabolites of PAHs, OH–PAHs such as 1–hydroxypyrene have been frequently used as indicators of human exposure to atmospheric PAHs (Jongeneelen, 2001; Srogi, 2007; Boogaard, 2008; Zhou et al., 2009). Hence, several proce–dures have been described to determine urine levels of OH–PAHs (Xu et al., 2004; Romanoff et al., 2006; Mattarozzi et al., 2009; Rossella et al., 2009; Wenger et al., 2009).

Among the few attempts to determine OH–PAHs in airborne particles, methods such as gas chromatography (GC) with mass spectrometry (MS) (Galceran et al., 1995; Romanoff et al., 2006; Wang et al., 2007; Mattarozzi et al., 2009) or electron capture (ECD) have been successful (Galceran et al., 1995). However, high–performance liquid chromatography (HPLC) has only rarely been used with ECD (Galceran and Moyano, 1995). HPLC/FL separation procedures (Kishikawa et al., 2004) are usually sensitive and selective and do not generally require a preliminary derivatization step making them relatively simple and quick. In contrast, GC

requires the prior conversion of OH–PAHs into volatile forms (Romanoff et al., 2006; Mattarozzi et al., 2009) and this deriva–tization procedure complicates the method and is also susceptible to contamination and analyte losses.

Our main objective in the present study was to develop a simple and sensitive analytical method with HPLC/FD to detect two OH–PAHs in atmospheric aerosols (Table 1). These compounds were selected as analytes because their parent PAHs are relatively abundant in airborne particles and because they are often used as bioindicators of human exposure to PAH's (Jongeneelen, 2001; Rossella et al., 2009; Zhou et al., 2009).

Table 1. *Hydroxy–PAHs determined by chromatography*

Compound	Abbreviation	Structure
1–hydroxypyrene	1–OHPYR	
2–hydroxyphenantrene	2–OHPH	

After establishing the optimal chromatographic conditions and calculating quality parameters, we detected OH–PAHs at levels of pg m^{-3} in aerosol samples obtained over an entire year in the city of Madrid (central Spain). In the second part of this study, we tried to correlate the data obtained with several physical (temperature, atmospheric pressure etc.) and chemical factors (NO, NO_2, etc.) related to atmospheric pollution using multivariate data analysis methods.

2. Experimental

2.1. Chemicals

The 1–hydroxypyrene (1–OHPYR) and 2–hydroxy–phenanthrene (2–OHPH) standards used were from Dr. Ehrenstorfer GMbH, 10 ng μL^{-1} in acetonitrile. Analytical–reagent grade dichloromethane (from Carlo Erba 99.9%), acetone, hexane, cyclohexane (from Carlo Erba 99.5%) were used to extract the organic compounds from atmospheric aerosols. For the mobile phase, HPLC–grade acetonitrile (99%), methanol (Carlo Erba 99.85%), acetic acid (from Carlo Erba 99.8%) and deionized water were used. For clean–up assays we used silica gel and 450 mg Alumina (neutral) MiniSpeed cartridges (Applied Separations).

2.2. Chromatographic apparatus

The equipment used was an Agilent Series 1200 Liquid Chromatograph equipped with an Eclipse XDB C–18 (Agilent) column (150 x 4.6 mm, 5 µm), maintained at 36.7°C with a Jones Chromatography 7971 temperature controller, a quaternary pump Agilent 1200 and a series 1100 fluorescence detector connected to a Chemstation computer system. The mobile phase was initially a mixture of acetonitrile/water (60/40) and the flow rate was 1.0 mL min^{-1}, and the separation was performed isocratically and the injection volume was 25 µL. Fluorescence was detected at wavelengths of 259/386 nm for 2–OHPH and 242/388 nm for OH–PYR.

2.3. Sample collection

The sampling site selected was the CIEMAT research centre (Centro de Investigaciones Energeticas, Medioambientales y Tecnologicas), 7 Km northwest of Madrid's city centre. Fine particulate (PM_{10}) air pollution samples were collected on previously heat–treated (400 °C for 24 hours) Whatman glass fiber filters using a high volume MCV CAV–A/mb sampler. After collection, the filters were wrapped separately in aluminum foil and stored in a freezer at –20 °C until analysis. The volume of ambient air drawn through the filter over 24 hours of sampling was about 550 m^3. From February 2008 to February 2009, we collected 55 samples of 550 m^3 (one sample per week).

2.4. Optimizing conditions of extraction, clean–up and chromatography

Initial experiments were designed to establish the best extraction procedure. To improve efficiency and reduce the amount of solvent and time needed for extraction, the effects of several factors including mobile phase composition and analysis time were explored as indicated below. Each experiment was run at least in triplicate.

To extract the OH–PAHs, we assessed the techniques Soxhlet (SX), microwave–assisted extraction (MAE) and ultrasound–assisted solvent extraction (USE). To find the best solvent, analyte recovery tests were performed with the OH–PAHs standards. OH–PAHs were added prior to extraction by applying about 10–25 µg L^{-1} of standard compounds to clean glass fiber filters and then cutting the labeled filters into pieces before testing the extraction procedure. For SX extraction, 200 mL solvent volumes were applied to the loaded filters for 8 hours. USE was performed twice using 10.0 mL of solvent for 30 minutes. Extraction using MAE consisted of subjecting the filters once for 40 min to a temperature gradient in 15.0 mL of solvent. The solvents tested were dichloromethane (DCM), acetone, hexane, methanol (MeOH) and different combinations of these.

For the SX extraction method, good results were obtained for 2–OHPH (above 70% recovery) using DCM as the solvent whereas the remaining solvents and combinations tested returned an extraction efficiency close to null. For 1–OHPYR, efficiencies were null (<2%) for all the solvents and their combinations tested and neither were good results observed for the MAE procedure.

For both compounds, best results were obtained when the OH–PAHs were ultrasonically extracted in closed tubes using MeOH as the solvent. We also observed that re–extraction failed to improve efficiency while this efficiency was increased when extraction was conducted at low temperature and the tubes were placed in a freezer post–extraction. For the extraction method optimization, were repeated the analysis with standards under the same conditions to test the repeatability. Extraction yields obtained were similar, with a standard deviation of 2–3% (n = 10). Figure 1 shows the recovery rates of OH–PAHs using MeOH and DCM in closed tubes at low temperature and then transferring the vessels to a freezer for 15 min. For 1–OHPYR, maximal extraction efficiencies were recorded using MeOH for 30 min. Actually, hydroxylated PAHs are less stable than their parent PAHs and are easily degraded when ambient temperature is high.

Filter extracts were concentrated to 0.5 mL under nitrogen flow and then cleaned using MiniSpe–ed cartridges. Two clean up steps using the cartridges, octadecyl C_{18} 18% and silica gel were tested. The solvents used to elute the extracts were hexane, DCM, MeOH and acetone. The MiniSpe–ed cartridges were previously conditioned with 2.0 mL hexane or 2.0 mL DCM. Next, the cartridges were loaded with the standards and the compounds were eluted. The analysis results showed no matrix effect on the OH–PAHs determination in airborne particulates, and there was no substantial improvement in the results, so the proposed method is shortened if dispensed of this stage.

To optimize the chromatographic conditions, the factors examined were: detection wavelength, injection volume, column temperature and the mobile phase and gradients. To optimize the mobile phase, different chromatographic methods were tested using different gradient programs and varying the solvents used

and analysis times. The use of acetonitrile/water in gradient mode provided good selectivity and sensitivity values for an analysis time of 12 min. When methanol/water was used, the method's sensitivity for detecting 1–OHPYR increased considerably, yet sensitivity towards 2–OHPH was reduced and the analysis time was lengthened to 17 min. Finally, we tested the use of the phase acetonitrile/water, 60/40 in isocratic mode, adding as buffer 1% acetic acid (99.8%) to adjust the pH, and managed to improve the sensitivities and detection limits, shortening the analysis time to 7 min.

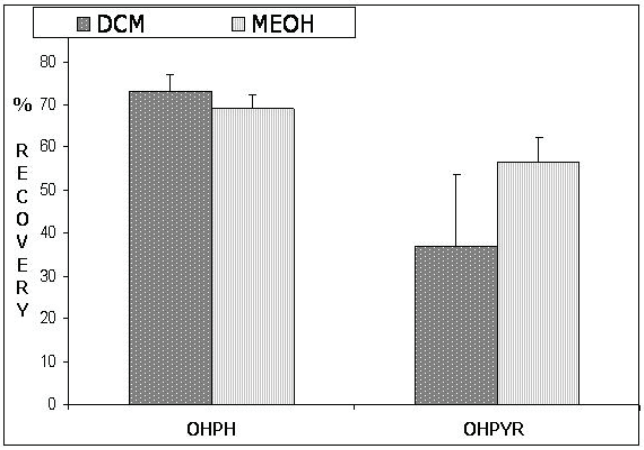


Figure 1. *Recovery rates of OH–PAHs using MeOH and DCM in closed tubes at low temperature.*

Calibration curves were prepared with OH–PAHs standards. Limits of detection, calculated as three times the standard deviation obtained for the blank, were 1.0 ng mL^{-1} for both 1–OHPYR and 2–OHPH covering the linear dynamic range of 5.0 to 500.0 ng mL^{-1} for both compounds with a relative standard deviation (n = 4) of 4.0%.

2.5. Sample preparation

The filters were cut into small pieces to extract the compounds by low–temperature USE in closed containers containing 10 mL of methanol (30 min). Another advantage of the proposed method is that there are no analyte losses during sample preparation. This was confirmed in assays performed using standards and airborne samples labeled with internal standards. In all these assays, recovery rates were always over 99%.

After the samples were left to stand for 15 min in a freezer, the extracts were filtered (PTFE, 0.22 µm) and the solvent was evaporated under a nitrogen flow. Finally, the extracts were re–suspended in 1.0 mL of methanol and directly injected into the HPLC system. Field blank filters were subjected to the same procedure to confirm a lack of significant contamination. Figure 2 represents typical chromatograms obtained for the extracts from airborne particulates and standard.

Due to a lack of reference materials, and in order to confirm each peak in the chromatograms for the extracts from airborne particulates eluted at the same time of the corresponding standard OH–PAHs, method validation was carried out using the emission and excitation spectra (Kishikawa et al., 2004). As shown in Figure 3, the excitation and emission spectra of the OH–PAHs in airborne particulates were in good agreement with those obtained from the standard solutions.

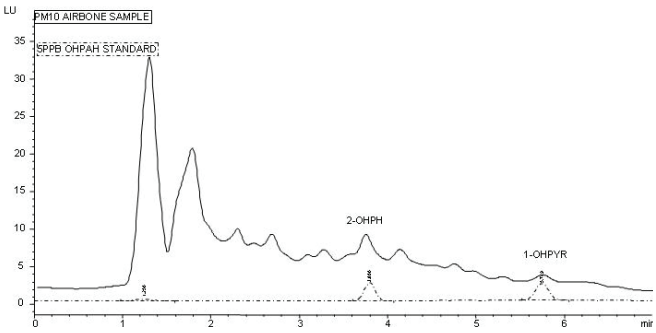


Figure 2. *Typical chromatograms obtained for the extracts from airborne particulates and calibration standard.*

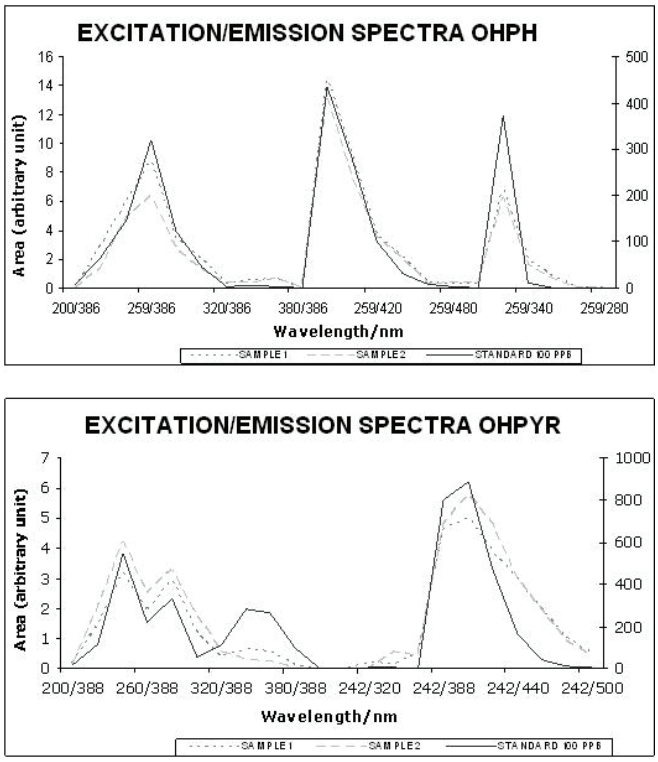


Figure 3. *Excitation and emission spectra of the OH–PAHs in airborne particulates and in standards.*

3. Results and Discussion

3.1. OH–PAHs in airborne particles determined over a one–year period

The proposed method was used to determine the two OH–PAHs in airborne particulate matter samples collected over a year. Table 2 provides the levels of the compounds recorded weekly from January 2008 to February 2009, along with another series of controlled variables recorded at the meteorological station at Isaac Peral 21, Madrid (longitude: 3° 43´ 0.16´´, latitude 40° 26´24.49´´, altitude 655 m).

In literature, there are not any studies conducted in Spain about the annual evolution of OH–PAHs and their relation with meteorological factors and other air pollutants. In general, the OH–PAH concentrations presented in this paper are in good agreement to those reported by other authors (Galceran and Moyano 1995; Kishikawa et al., 2004; Wang et al., 2007), observed in pg m^{-3} order, and more abundant in cold seasons than in warm seasons.

Table 2. *Data obtained for the 55 atmospheric aerosol samples collected over one year*

Sample	Date	2–OHPH	1–OHPYR	T	P	RH	SR	$PM_{2.5}$	PM_{10}	O_3	NO	NO_2	N_2O_x	SO_2
1	28/01/2008	180.2	83.5	9.6	936.2	52.6	136.0	24.9	40.3	8.2	143.9	123.3	267.2	26.0
2	30/01/2008	115.9	66.7	8.8	933.7	55.3	124.4	28.0	51.8	10.6	149.2	126.1	275.3	26.4
3	05/02/2008	144.7	43.0	7.2	931.8	78.9	124.6	20.6	32.9	12.6	91.6	89.9	181.5	13.7
4	07/02/2008	244.1	13.5	12.1	936.7	60.5	146.1	15.9	22.7	18.7	81.5	93.2	174.7	19.6
5	13/02/2008	43.1	17.7	8.4	931.1	54.2	91.7	12.1	11.6	39.2	16.3	67.0	83.3	17.7
6	20/02/2008	136.8	44.5	11.7	926.6	80.2	80.0	19.4	22.0	12.6	71.3	90.7	161.9	11.5
7	28/02/2008	59.6	16.8	12.6	928.2	70.9	111.9	15.4	20.1	15.2	69.6	84.3	153.8	11.4
8	03/03/2008	46.8	14.8	14.4	926.0	54.6	178.3	15.8	40.5	28.9	69.9	73.2	143.2	14.6
9	11/03/2008	89.1	29.8	15.1	924.1	49.0	165.0	11.2	18.7	40.6	28.7	48.7	77.3	9.0
10	17/032008	27.9	7.1	13.0	921.2	48.7	161.5	12.3	27.6	34.8	36.6	70.1	106.6	10.8
11	25/03/2008	28.9	8.0	12.5	922.1	48.1	184.8	12.0	20.6	38.8	42.8	66.9	109.7	9.9
12	02/04/2008	47.6	24.5	16.7	932.4	47.0	220.4	9.1	17.4	34.5	28.3	54.8	83.1	9.7
13	15/04/2008	46.7	17.8	14.0	926.8	39.1	224.5	10.6	22.5	42.9	20.9	74.3	95.2	9.6
14	22/04/2008	35.5	8.5	13.9	923.9	50.6	240.0	11.7	22.4	42.3	29.3	63.9	93.2	7.5
15	29/04/2008	33.0	1.7	14.7	918.4	40.1	264.5	13.0	22.3	53.2	29.0	63.2	92.2	7.7
16	06/05/2008	21.1	8.2	22.5	923.3	42.1	198.0	16.0	36.6	40.3	19.1	68.9	88.0	8.3
17	12/05/2008	19.0	4.1	17.2	921.0	57.9	175.1	16.0	36.6	40.3	19.1	68.9	88.0	8.3
18	21/05/2008	27.1	6.6	17.2	921.0	57.9	175.1	16.8	25.2	23.9	44.8	75.7	120.5	8.4
19	29/05/2008	23.9	5.4	15.4	920.6	58.6	194.0	11.1	17.0	45.8	33.4	66.2	99.5	7.8
20	03/06/2008	19.6	5.2	18.9	925.7	45.0	247.3	11.6	14.7	56.9	7.2	44.7	51.9	7.1
21	12/06/2008	39.9	6.5	20.8	927.1	48.7	272.9	16.0	19.8	53.3	13.1	40.8	53.9	9.3
22	18/06/2008	36.3	6.9	22.8	925.4	41.6	315.3	15.3	27.0	45.5	21.3	69.3	90.6	10.1
23	26/06/2008	22.3	6.2	28.5	926.2	39.3	304.7	25.8	43.8	58.4	9.4	80.8	90.1	10.5
24	01/07/2008	20.8	6.5	28.1	923.9	32.3	305.7	19.8	37.6	54.1	13.1	86.4	99.4	10.9
25	09/07/2008	24.8	7.2	26.3	926.0	35.5	307.8	19.6	37.5	35.7	23.6	97.1	120.7	11.2
26	17/07/2008	22.6	6.4	25.9	926.1	40.8	282.7	18.6	32.7	54.1	10.4	76.8	87.1	11.0
27	22/07/2008	19.4	5.1	27.4	928.0	27.2	301.3	14.2	26.1	68.8	5.2	60.2	65.4	10.2
28	31/07/2008	28.1	6.3	27.1	925.4	30.0	283.6	17.0	31.7	40.3	24.5	79.7	104.2	10.8
29	06/08/2008	21.4	5.0	29.8	922.0	31.7	266.7	16.2	35.3	45.5	25.4	81.1	106.5	11.4
30	12/08/2008	17.9	4.9	24.2	918.3	53.1	260.6	6.7	14.8	32.5	18.1	36.0	54.1	10.2
31	20/08/2008	9.6	3.5	24.8	925.0	38.5	268.2	14.4	25.1	53.3	8.1	53.8	61.8	10.4
32	28/08/2008	24.1	5.2	27.4	926.2	44.4	228.9	14.6	24.6	43.7	9.5	54.6	64.1	10.7
33	02/09/2008	13.8	4.7	24.9	924.5	41.1	229.2	14.9	28.4	28.9	43.3	83.4	126.7	12.3
34	11/09/2008	13.6	4.8	22.3	922.5	50.0	217.2	13.7	26.5	22.4	44.5	60.9	105.4	11.8
35	17/09/2008	45.4	6.6	22.2	921.3	39.3	217.7	22.3	35.6	20.4	47.4	105.2	152.6	13.2
36	25/09/2008	13.6	5.4	17.5	927.2	44.0	200.8	13.9	20.5	47.9	6.6	47.7	54.3	10.7
37	30/09/2008	58.7	6.9	18.8	926.5	43.8	191.4	16.3	24.2	44.1	14.4	72.9	87.3	12.1
38	09/10/2008	82.9	16.8	14.7	929.1	44.0	166.4	8.3	10.3	40.5	7.1	33.7	40.7	11.3
39	16/10/2008	11.5	4.5	18.1	928.7	62.6	136.4	27.3	54.0	8.9	76.3	98.9	175.2	14.6
40	23/10/2008	98.6	21.3	12.1	927.7	46.0	95.8	11.4	23.0	41.0	25.3	52.0	77.2	14.6
41	29/10/2008	52.8	12.0	8.2	918.0	53.5	139.5	8.6	16.2	38.2	38.8	59.5	98.2	13.7
42	06/11/2008	104.1	45.7	9.5	926.8	69.3	109.1	19.6	18.3	15.3	80.4	85.1	165.5	16.1
43	12/11/2008	72.8	17.7	7.6	932.5	65.1	94.3	14.3	11.8	18.4	51.9	73.5	125.4	13.5
44	20/11/2008	156.4	72.6	9.9	930.1	59.3	113.4	12.3	9.9	34.4	26.2	50.6	76.8	16.7
45	24/11/2008	24.0	6.0	9.9	930.1	59.3	113.4	12.3	9.9	34.4	26.2	50.6	76.8	16.7
46	04/12/2008	38.9	14.4	6.9	920.1	87.7	34.5	17.9	20.4	17.9	86.7	79.6	166.3	15.7
47	18/12/2008	188.6	60.6	7.6	928.6	71.1	95.1	24.9	25.9	8.6	135.7	105.8	241.5	22.2
48	22/12/2008	247.3	121.0	8.7	935.2	58.4	100.8	30.2	40.0	6.7	207.3	135.1	342.4	39.5
49	07/01/2009	75.9	51.1	2.5	919.4	45.3	109.1	8.7	7.1	38.8	14.6	47.6	62.3	15.9
50	14/01/2009	140.9	53.5	2.8	926.6	91.4	45.5	26.7	22.6	9.4	105.0	106.7	211.6	17.0
51	21/01/2009	33.0	9.2	5.2	920.6	49.3	104.7	8.8	10.7	44.4	34.5	60.5	95.0	12.3
52	28/01/2009	13.2	3.1	10.8	924.7	84.5	90.4	9.8	10.8	29.3	37.9	49.9	87.7	12.7
53	11/02/2009	78.7	23.1	8.7	930.9	49.4	90.4	12.6	11.9	35.2	42.2	63.4	105.6	12.7
54	19/02/2009	118.4	36.0	10.2	925.8	50.0	150.0	17.2	28.8	15.7	90.2	104.1	194.2	25.2
55	26/02/2009	92.8	30.6	10.2	925.8	50.0	150.0	17.2	28.8	15.7	90.2	104.1	194.2	25.2

2–OHPH and 1–OHPYR (pg m^{-3}), T = ambient temperature (°C), P = atmospheric pressure (mb), RH = relative humidity (%), SR = solar radiation (Med h^{-1}), $PM_{2.5}$ and PM_{10} = particulate matter (μg), remaining variables expressed in (μg m^{-3})

Figure 4 shows the seasonal variation produced in the mean 1–OHPYR and 2–OHPH concentrations recorded for each month throughout the year. It may be noted that 1–OHPYR concen–trations were higher in winter (13.6–65.3 pg m^{-3}, average 33.7 pg m^{-3}) than summer (4.6–16.9 pg m^{-3}, average 7.6 pg m^{-3}) and 2–OHPH values showed the same variation (winter 48.2–158.2 pg m^{-3}, average 94.2 pg m–3; summer (18.3–43.3 pg m^{-3}, average 27.7 pg m^{-3}).

There is still no appropriate legislation and not enough studies about its impact on human health, but due to their potential toxicity, it is interesting to know what concentrations are inhaled.

3.2. Factor analysis

The covariance matrix with the 13 analyzed variables was constructed using normalized data and, therefore, coincides with the correlation matrix (Table 3). Several clear atmospheric–

chemical relationships could be readily inferred including high positive correlation (values in bold, r = 0.267 to 0.918) between 1–OHPYR and 2–OHPH, and between the two compounds and NO, NO_2, N_2O_x and SO_2 levels along with atmospheric pressure, solar radiation, etc. The highest correlation was the one observed between the levels of the two compounds. Positive correlation was detected between the OH–PAHs and the nitrogen and sulfur oxides, while increases in ozone, temperature and radiation were linked to a reduction in hydroxylate concentrations. These findings seem to confirm the conclusions drawn about the formation of hydroxylated compounds in the atmosphere via photochemical reactions. Moreover, the purported relationship between airborne OH–PAH in fine particulate matter and estrogenic activity emphasizes the potential interest of yearly monitoring of these compounds. The Bartlett chi–squared statistic of Bartlett's sphericity test was 1 117.6 (the critical value is 99.6 for 78 degrees of freedom at the 95% significance level, p = 0.000), indicating that the variables examined are not orthogonal but correlated. This means that data variability could be explained with a lower number of variables (or principal components, PC).

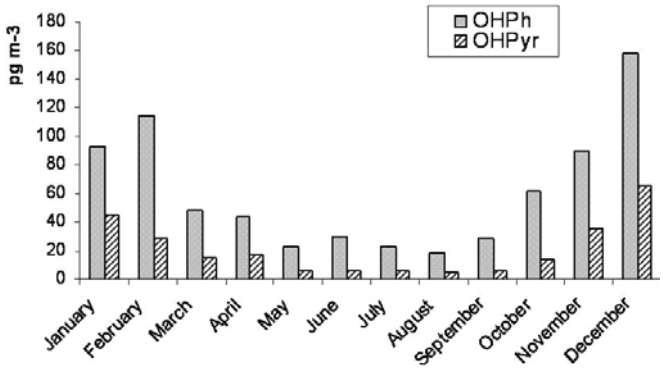


Figure 4. *The seasonal variation produced in the mean 1–OHPYR and 2–OHPH concentrations recorded for each month throughout the year.*

For the factor analysis, the correlation matrix was diagonalized and new factors (PCs) were obtained by linearly combining the original variables. PCs with eigenvalues greater than 1 must be preserved to comprehend the underlying data structure (Jackson, 1991). In our case, there were three such PCs, which are shown in Table 4. PC1 is able to explain 56.7% of the variance and most variables contributed to this component, although temperature, solar radiation and O_3 did so in a positive manner and the remaining factors, i.e., the indicators of chemical pollution contributed negatively to this component. Accordingly, this component could be assigned the "chemical pollution" effect. The second component could explain 20.9% of the variance and was positively contributed by relative humidity (RH) and negatively by temperature and solar radiation. PC2 could therefore be assigned the effect "seasonality".

If we represent the values of each component that each item takes for each new variable as ''scores'', a dimensional graph is obtained that clearly illustrates these contributions. Figure 5 shows the representation of ''score 2'' versus ''score 1''. In the same plot, the "loadings" of the factors are also indicated. According to these loadings, the more to the left a sample appears (factor 1 negative) the greater is its extent of chemical pollution, and samples showing a similar amount of pollution will appear higher or lower (factor 2 positive or negative) according to the time of the year they were collected.

This figure complements in two dimensions the seasonality results observed before in Figure 4. It can be seen that samples showing the greater OH–PAH concentrations, i.e., samples 1, 2, 3, 4, 6 corresponding to January and February and samples 42, 44, 47, 48 (off–scale), 50 and 54, obtained in November and December appear grouped in the left quadrants, while samples with lower concentrations appear more towards the right. It may also be noted that samples corresponding to the summer months appear below those corresponding to autumn and spring months.

3.3. Cluster analysis

Through cluster analysis, a dendrogram was produced clustering the variables (Figure 6a). Confirming the results of the factor analysis, we observed a cluster including the "seasonal" factors ozone, temperature and solar radiation. 1–OHPYR and 2–OHPH appeared in the same group close to another group with the "pollution" factors nitrogen and sulfur oxides. Finally, a fourth group emerged containing the different particles PM_{10}–$PM_{2.5}$ but also encompassing NO_2. Although the latter is difficult to explain, it confirms our previous observations.

When clusters were constructed on the observations using the Ward method and Euclidean distances, the same four clusters appeared (Figure 6b). These clusters were practically identical to the ones provided in Figure 5. Thus, to the left (group 1) appear the samples most affected by the OH–PAHs and the chemical pollutants. It may also be observed that the least polluted samples group together according to the season during which they were collected.

Table 3. *Correlation matrix for the 13 physical/chemical factors recorded*

	2–OH PH	1–OH PYR	T	P	RH	SR	$PM_{2.5}$	PM_{10}	O_3	NO	NO_2	N_2Ox	SO_2
2–OHPH	1												
1–OHPYR	**0.834**	1											
T	**–0.559**	**–0.555**	1										
P	**0.620**	**0.524**	–0.229	1									
RH	**0.418**	**0.365**	**–0.676**	0.173	1								
SR	**–0.532**	**–0.518**	**0.882**	**–0.267**	**–0.782**	1							
$PM_{2.5}$	**0.428**	**0.492**	0.010	**0.398**	0.228	–0.105	1						
PM_{10}	0.047	0.122	**0.384**	0.151	–0.192	**0.291**	**0.758**	1					
O_3	**–0.619**	**–0.592**	**0.559**	**–0.367**	**–0.686**	**0.679**	**–0.511**	–0.212	1				
NO	**0.745**	**0.778**	**–0.524**	**0.458**	**0.529**	**–0.548**	**0.683**	**0.379**	**–0.837**	1			
NO_2	**0.539**	**0.551**	–0.172	**0.377**	0.225	–0.220	**0.854**	**0.673**	**–0.668**	**0.812**	1		
N_2Ox	**0.703**	**0.730**	**–0.419**	**0.449**	**0.441**	**–0.452**	**0.778**	**0.504**	**–0.813**	**0.977**	**0.918**	1	
SO_2	**0.741**	**0.801**	**–0.483**	**0.537**	0.283	**–0.498**	**0.549**	0.257	**–0.649**	**0.840**	**0.682**	**0.820**	1

r (critical)= 0.266

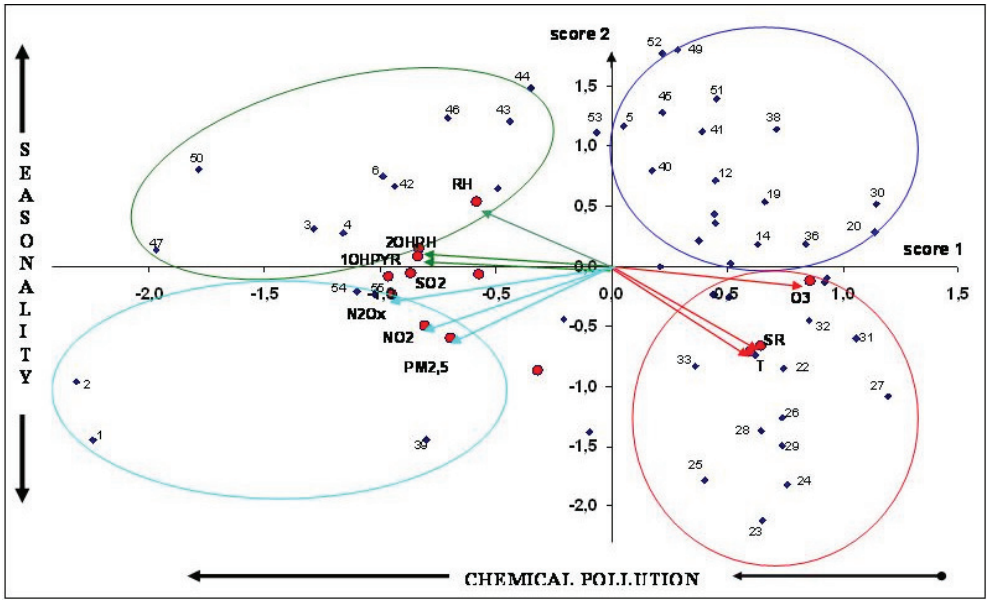


Figure 5. *Scores of urban aerosol samples (PM_{10}) on the bi–dimensional plane defined by the first two factors.*

Table 4. *Factor loadings and eigenvalues of the first three principal factors*

Variable	PC1	PC2	PC3
2–OHPH	**–0.828**	0.147	**–0.379**
1–OHPYR	**–0.837**	0.084	**–0.335**
T	**0.602**	**–0.713**	–0.039
P	**–0.571**	–0.067	**–0.589**
RH	**–0.581**	**0.540**	**0.442**
SR	**0.650**	**–0.666**	–0.173
$PM_{2.5}$	**–0.694**	**–0.597**	0.160
PM_{10}	**–0.315**	**–0.868**	0.209
O_3	**0.861**	–0.128	**–0.299**
NO	**–0.961**	–0.083	0.085
NO_2	**–0.803**	**–0.492**	0.160
N_2O_x	**–0.947**	–0.238	0.116
SO_2	**–0.866**	–0.059	–0.250
Eigenvalue	7.374	2.713	1.097
%Variance	56.7	20.9	8.4

4. Conclusions

A simple and sensitive analytical method with HPLC–FDL is proposed to determine the OH–PAHs, 1–hydroxypyrene (1–OHPYR) and 2–hydroxyphenantrene (2–OHPH) in concentrations of the order of pg m^{-3}, applicable to the analysis of atmospheric aerosol samples. Monitoring of airborne samples collected in Madrid over an entire year indicated the levels of these compounds varied from 1.7 pg m^{-3} to 244.1 pg m^{-3}, showing clear seasonal behavior. Using several statistical tools, this behavior was confirmed and correlations identified between 2–OHPH and 1–OHPYR concen–trations and those of chemical factors, mainly nitrogen and sulfur oxides, involved in photochemical reactions. In contrast, as temperature and solar radiation rise, ozone levels also increase reducing the OH–PAHs levels.

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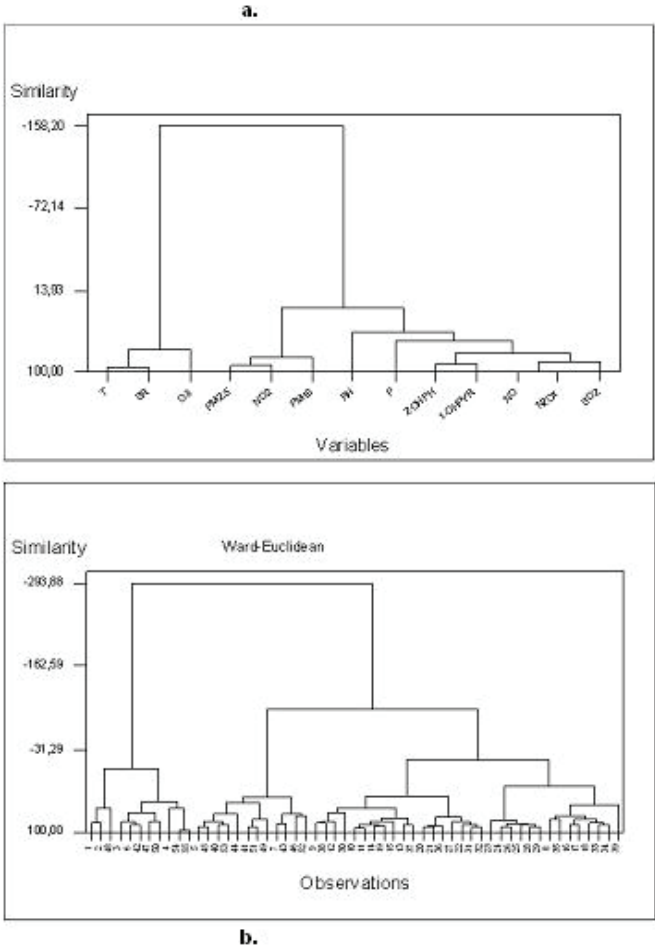

Figure 6. *Dendrograms showing the variables determined in the different samples and observations made.*

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